APPLICATION OF PHAGE DISPLAY AND PLASMID DISPLAY TO BROADEN THE SPECIFICITY OF HUMAN FBS1 FOR CAPTURE OF N-GLYCOSYLATED PEPTIDES

James C Samuelson, New England Biolabs, USA

The objective of this study was to develop a method for selective and comprehensive enrichment of N-linked glycopeptides to facilitate biomarker discovery. The natural function of human Fbs1 is to bind misfolded N-linked glycoproteins by recognition of the common pentasaccharide core motif (Man3GlcNAc2) of the N-glycan. We show that Fbs1 is able to bind diverse types of N-linked glycomolecules, however, wild-type Fbs1 preferentially binds high mannose containing glycans. To reduce the bias during N-glycomolecule enrichment experiments, we isolated Fbs1 variants with altered specificity through mutagenesis and plasmid display selection. Five cycles of E. coli propagation and in vitro panning against immobilized fetuin resulted in a pool of variants with improved binding for complex N-glycopeptides. The most valuable Fbs1 variant enabled substantially unbiased N-glycopeptide enrichment from a level of 3.5% to 66% when applied to IgG-depleted serum. Importantly, plasmid display is a rapid method for altering substrate binding specificity that is an attractive alternative to phage display.

M13 phage display of Fbs1 was also accomplished by non-standard methodology. Since Fbs1 folding is impaired by disulfide bond formation in the E.coli periplasm, a mutant E.coli host was critical for proper display on the surface of M13 phage. Furthermore, display of functional Fbs1 could only be achieved by expressing the pIII-Fbs1 fusion protein with an SRP-dependent signal peptide.

Significance:

The Fbs1 study revealed that plasmid display is a powerful alternative to phage display. In particular, plasmid display is appropriate for non-secretory proteins. The plasmid display selection process is very rapid as 5 cycles may be performed in 5 days. Our efforts to display Fbs1 by M13 phage display were met by complication. In response, we developed a method capable of functional display of non-secretory protein on the surface of M13 phage.

Reference: Chen M and Samuelson JC “A DsbA-deficient Periplasm Enables Functional Display of a Protein with Redox-Sensitive Folding on M13 Phage” Biochemistry (2016) 55(23):3175-9.